

=> S HYDROLASE;S LIPASE;S ESTERASE;S PROTEASE;S STEREO?

16971 HYDROLASE

7539 HYDROLASES

L1 20821 HYDROLASE
(HYDROLASE OR HYDROLASES)

39211 LIPASE

7370 LIPASES

L2 40443 LIPASE
(LIPASE OR LIPASES)

28264 ESTERASE

8313 ESTERASES

L3 30717 ESTERASE
(ESTERASE OR ESTERASES)

74373 PROTEASE

27205 PROTEASES

L4 86597 PROTEASE
(PROTEASE OR PROTEASES)

L5 207089 STEREO?

=> S MUTATION

182400 MUTATION

117126 MUTATIONS

L6 226352 MUTATION
(MUTATION OR MUTATIONS)

=> S L6 AND L5

L7 641 L6 AND L5

=> S L7 AND L1;S L7 AND L2;S L7 AND L3;S L7 AND L4

L8 14 L7 AND L1

L9 17 L7 AND L2

L10 7 L7 AND L3

L11 10 L7 AND L4

=> S L8,L9,L10,L11

L12 40 (L8 OR L9 OR L10 OR L11)

=> D 1-40 CBIB ABS

L12 ANSWER 1 OF 40 CAPLUS COPYRIGHT 2003 ACS

2003:243340 Cellular and molecular characterization of the adipose phenotype of the aromatase-deficient mouse. Misso, Marie L.; Murata, Yoko; Boon, Wah Chin; Jones, Margaret E. E.; Britt, Kara L.; Simpson, Evan R. (Prince Henry's Institute of Medical Research, Clayton, 3168, Australia). Endocrinology, 144(4), 1474-1480 (English) 2003. CODEN: ENDOAO. ISSN: 0013-7227. Publisher: Endocrine Society.

AB Estrogen deficiency in the aromatase knockout (ArKO) mouse leads to the development of obesity by as early as 3 mo of age, which is characterized by a marked increase in the wts. of gonadal and infrarenal fat pads. Humans with natural ***mutations*** of the aromatase gene also develop a metabolic syndrome. In the present study cellular and mol. parameters were investigated in gonadal adipose tissue from 10-wk-old wild-type (WT) and ArKO female mice treated with 17 β -estradiol or placebo to identify the basis for the increase in intraabdominal obesity.

• . ***Stereol*** . examn. revealed that adipocytes isolated from ArKO mice were significantly larger and more abundant than adipocytes isolated from WT mice. Upon treatment with estrogen, the vol. of these adipocytes was greatly reduced, whereas the redns. in the no. of adipocytes was much less pronounced. Transcriptional anal. using real-time PCR revealed concomitant changes with adipocyte vol. in the levels of transcripts encoding leptin and lipoprotein ***lipase***, whereas peroxisome proliferator-activated receptor .gamma. levels followed a pattern closer to that of adipocyte no. Little change was obsd. in levels of transcripts for factors involved in de novo fatty acid synthesis, .beta.-oxidn., and lipolysis, suggesting that changes in the uptake of lipids from the circulation are the main mechanisms by which estrogen regulates lipid metab. in these mice.

L12 ANSWER 2 OF 40 CAPLUS COPYRIGHT 2003 ACS

2002:885757 Document No. 138:134569 Hereditary deafness and phenotyping in humans. Bitner-Blindzicz, Maria (Unit of Clinical and Molecular Genetics, Institute of Child Health, London, UK). British Medical Bulletin, 63, 73-94 (English) 2002. CODEN: BMBUAQ. ISSN: 0007-1420. Publisher: Oxford University Press.

AB A review. Hereditary deafness has proved to be extremely heterogeneous genetically with more than 40 genes mapped or cloned for non-syndromic dominant deafness and 30 for autosomal recessive non-syndromic deafness. In spite of significant advances in the understanding of the mol. basis of hearing loss, identifying the precise genetic cause in an individual remains difficult. Consequently, it is important to exclude syndromic causes of deafness by clin. and special investigation and to use all available phenotypic clues for diagnosis. A clin. approach to the etiol. investigation of individuals with hearing loss is suggested, which includes ophthalmol. review, renal ultrasound scan and neuro-imaging of petrous temporal bone. Mol. screening of the GJB2 (Connexin 26) gene should be undertaken in all cases of non-syndromic deafness where the cause cannot be identified, since it is a common cause of recessive hearing impairment, the screening is straightforward, and the phenotype unremarkable. By the same token, mitochondrial inheritance of hearing loss should be considered in all multigeneration families, particularly if there is a history of exposure to amino-glycoside antibiotics, since genetic testing of specific mitochondrial genes is tech. feasible. Most forms of non-syndromic autosomal recessive hearing impairment cause a prelingual hearing loss, which is generally severe to profound and not assocd. with abnormal radiol. Exceptions to this include DFNB2 (MYO7A), DFNB8/10 (TMPRSS3) and DFNB16 (STRC) where age of onset may sometimes be later on in childhood, DFNB4 (SLC26A4) where there may be dilated vestibular aqueducts and endolymphatic sacs, and DFNB9 (OTOF) where there may also be an assocd. auditory neuropathy. Unusual phenotypes in autosomal dominant forms of deafness, include low frequency hearing loss in DFNA1 (HDIA1) and DFNA6/14/38 (WFS1), mid-frequency hearing loss in DFNA8/12 (TECTA), DFNA13 (COL11A2) and vestibular symptoms and signs in DFNA9 (COCH) and sometimes in DFNA11 (MYO7A). Continued clin. evaluation of types and course of hearing loss and correlation with genotype is important for the intelligent application of mol. testing in the next few years.

L12 ANSWER 3 OF 40 CAPLUS COPYRIGHT 2003 ACS

2002:740435 Document No. 138:1598 Modification of the enantioselectivity of two homologous thermophilic carboxylesterases from *Alicyclobacillus acidocaldarius* and *Archaeoglobus fulgidus* by random mutagenesis and screening. Manco, Giuseppe; Carrea, Giacomo; Giosue, Elena; Ottolina, Gianluca; Adamo, Giovanna; Rossi, Mose (Istituto di Biochimica delle Proteine, CNR, Naples, 80125, Italy). Extremophiles, 6(4), 325-331 (English) 2002. CODEN: EXTRFI. ISSN: 1431-0651. Publisher: Springer-Verlag Tokyo.

AB The ***esterase*** genes est2 from *Alicyclobacillus acidocaldarius* and AF1716 from *Archaeoglobus fulgidus* were subjected to error-prone PCR in an effort to increase the low enantioselectivity of the corresponding enzymes EST2 and AFEST, resp. The model substrate (RS)-p-nitrophenyl-2-chloropropionate was chosen to produce (S)-2-chloropropionic acid, an important intermediate in the synthesis of some optically pure compds., such as the herbicide mecoprop. In the case of EST2, a single mutant, Leu212Pro, was obtained showing a slightly enhanced preference toward the (S) substrate; in the case of AFEST, a double mutant, Leu101Ile/Asp117Gly,

was obtained showing an increased preference in the opposite direction. The 3-D structures of the EST2 and AFEST enzymes were analyzed by mol. modeling to det. the effects of the ***mutations*** .

Mutations were positioned differently in the structures, but in both cases caused small modifications around the active site and in the oxyanion loop.

L12 ANSWER 4 OF 40 CAPLUS COPYRIGHT 2003 ACS

2002:629322 Document No. 137:347267 ***Mutation*** of the novel gene Tmie results in sensory cell defects in the inner ear of spinner, a mouse model of human hearing loss DFNB6. Mitchem, Kristina L.; Hibbard, Ellen; Beyer, Lisa A.; Bosom, Ken; Dootz, Gary A.; Dolan, David F.; Johnson, Kenneth R.; Raphael, Yehoash; Kohrman, David C. (Department of Otolaryngology/Kresge Hearing Research Institute, University of Michigan Medical School, Ann Arbor, MI, 48109, USA). Human Molecular Genetics, 11(16), 1887-1898 (English) 2002. CODEN: HMGE5. ISSN: 0964-6906.

Publisher: Oxford University Press.

AB The recessive ***mutation*** at the mouse spinner (sr) locus results in hearing loss and vestibular dysfunction due to neuroepithelial defects in the inner ear. Using a positional cloning strategy, we have identified the mutant locus responsible for this pathol. The affected gene (Tmie) lies within a 40 kb deletion in the original sr allele. In a newly identified allele, Tmie contains a nonsense ***mutation*** expected to truncate the C-terminal end of its product. The 153 amino acid protein encoded by the gene shows no similarity to other known proteins, and is predicted to contain a signal peptide and at least one transmembrane domain. Tmie transcripts were identified in several tissues, including the cochlea. Loss of function of Tmie results in postnatal alterations of sensory hair cells in the cochlea, including defects in ***stereocilia***, the apical projections of hair cells that are important in mechano-transduction of sound. These morphol. defects are assocd. with a profound failure to develop normal auditory function. Consistent with a conserved role for this gene in the cochlea, the genetic mapping data presented here support human TMIE as the gene affected at DFNB6, a non-syndromic hearing loss locus. The spinner mutant is thus a valuable model for insight into mechanisms of human deafness and development of sensory cell function.

L12 ANSWER 5 OF 40 CAPLUS COPYRIGHT 2003 ACS

2002:419347 Document No. 137:59328 Fluorescence spectroscopic studies on structure and function of lipolytic enzymes. Hermetter, Albin; Mayer, Birgit; Scholze, Hubert; Zenzmaier, Elfriede; Graupner, Marion (SFB-Biocatalysis, Department of Biochemistry and Food Chemistry, Technical University Graz, Graz, Austria). Supramolecular Structure and Function 7, [Proceedings of the International Summer School on Biophysics], 7th, Rovinj, Croatia, Sept. 14-25, 2000, Meeting Date 2000, 63-74. Editor(s): Pifat-Mrzljak, Greta. Kluwer Academic/Plenum Publishers: New York, N. Y. ISBN: 0-306-46672-4 (English) 2001. CODEN: 69CQ07.

AB A review. Activity and ***stereoselectivity*** of lipolytic enzymes were investigated by fluorescence spectroscopy. ***Mutations*** in the close vicinity of the catalytic triad of recombinant ***lipases*** from *Rhizopus oryzae* gave rise to dramatic changes in activity and ***stereoselectivity*** towards the long-chain alkyldiacylglycerols. Phys. properties of lipolytic enzymes in soln. were investigated by Trp fluorescence. Lipid-protein interactions were studied using active site-labeling by fluorescent inhibitors. The effect of lipid structure and configuration was investigated on lipid-protein interactions.

L12 ANSWER 6 OF 40 CAPLUS COPYRIGHT 2003 ACS

2002:290229 Document No. 136:352007 Leukotriene A4 ***hydrolase*** : Selective abrogation of leukotriene B4 formation by ***mutation*** of aspartic acid 375. Rudberg, Peter C.; Tholander, Fredrik; Thunnissen, Marjolein M. G. M.; Samuelsson, Bengt; Haeggstrom, Jesper Z. (Department of Medical Biochemistry and Biophysics, Division of Chemistry II, Karolinska Institutet, Stockholm, S-171 77, Swed.). Proceedings of the National Academy of Sciences of the United States of America, 99(7), 4215-4220 (English) 2002. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.

AB Leukotriene A4 (LTA4) ***hydrolase*** (I) is a bifunctional zinc Zn metalloenzyme that catalyzes the final and rate-limiting step in the

biosynthesis of leukotriene B4 (LTB4), a classical chemoattractant and immune-modulating lipid mediator. Two chem. features are key to the bioactivity of LTB4, namely, the chirality of the 12R-OH group and the cis-trans-trans geometry of the conjugated triene structure. From the crystal structure of I, a hydrophilic patch composed of Gln-134, Tyr-267, and Asp-375 was identified in a narrow and otherwise hydrophobic pocket, believed to bind LTA4. In addn., Asp-375 belonged to peptide K21, a previously characterized 21-residue active site-peptide to which LTA4 binds during suicide inactivation. Here, the authors used site-directed mutagenesis and x-ray crystallog. to show that Asp-375, but none of the other candidate residues, was specifically required for the epoxide ***hydrolase*** activity of I. Thus, ***mutation*** of Asp-375 led to a selective loss of the ability of I to generate LTB4, whereas the aminopeptidase activity was preserved. The authors propose that Asp-375, possibly assisted by Gln-134, acts as a crit. determinant for the ***stereoselective*** introduction of the 12R-OH group and thus the biol. activity of LTB4.

L12 ANSWER 7 OF 40 CAPLUS COPYRIGHT 2003 ACS

2002:79603 Document No. 136:212374 Crown ether strategy toward chemical activation of biological protein functions. Tsukube, Hiroshi; Yamada, Takashi; Shinoda, Satoshi (Department of Chemistry, Graduate School of Science, Osaka City University, Osaka, 558-8585, Japan). Journal of Heterocyclic Chemistry, 38(6), 1401-1408 (English) 2001. CODEN: JHTCAD. ISSN: 0022-152X. Publisher: HeteroCorporation.

AB A review. The chem. activation of biol. proteins is outlined, in which small mols. are used to alter the chem. and phys. properties of biol. proteins through direct or indirect interactions. Crown ethers have the potential to modulate the protein functions by supramol. complexations, because they bind alkylammonium and other ionic residues of the proteins as well as ionic components in their systems. Two interesting examples are described in which crown ether derivs. improved the protein functions: (1) enhancement of reactivity and enantioselectivity in ***lipase***-catalyzed asym. reactions; and (2) generation of catalytic activity in the oxidn. with cytochrome c. This chem. activation based on crown ether chem. can be viewed as a complementary method to biol. ***mutation*** in modifying the biol. protein functions.

L12 ANSWER 8 OF 40 CAPLUS COPYRIGHT 2003 ACS

2001:928177 Document No. 136:179667 Complete reversal of enantioselectivity of an enzyme-catalyzed reaction by directed evolution. Zha, Dongxing; Wilensek, Stephanie; Hermes, Markus; Jaeger, Karl-Erich; Reetz, Manfred T. (Max-Planck-Institut fuer Kohlenforschung, Muelheim/Ruhr, D-45470, Germany). Chemical Communications (Cambridge, United Kingdom) (24), 2664-2665 (English) 2001. CODEN: CHCOFS. ISSN: 1359-7345. Publisher: Royal Society of Chemistry.

AB The combination of error prone PCR at high ***mutation*** rate and DNA shuffling can be used to invert the direction of enantioselectivity of a ***lipase***-catalyzed hydrolytic kinetic resoln. involving a chiral ester.

L12 ANSWER 9 OF 40 CAPLUS COPYRIGHT 2003 ACS

2001:874037 Document No. 136:185530 In vitro synthesis of cellulose and related polysaccharides. Kobayashi, Shiro; Sakamoto, Junji; Kimura, Shunsaku (Department of Materials Chemistry, Graduate School of Engineering, Kyoto University, Kyoto, 606-8501, Japan). Progress in Polymer Science, 26(9), 1525-1560 (English) 2001. CODEN: PRPSB8. ISSN: 0079-6700. Publisher: Elsevier Science Ltd..

AB A review with 170 refs. is presented. In vitro synthesis of cellulose (I) and related polysaccharides, in which polymer synthesis using an isolated enzyme as catalyst ('enzymic polymn.'), and conventional chem. synthesis are described. Typical natural polysaccharides, e.g. I, xylan, and chitin, having a .beta.(1.fwdarw.4) structure were successfully synthesized for the 1st time by using enzymic polymn. ***Hydrolases*** were most often employed as catalysts, which catalyze the bond-cleavage (hydrolysis) of polysaccharides in vivo, but the glycosidic bond-formation in vitro, to give polysaccharides. All enzymic polymns. gave a polysaccharide having a structure with perfect control of regio- and ***stereo***-selectivity. A key issue for the polymn. to occur is the appropriate design of monomers which is considered to be close in structure to that of the hydrolysis transition state. This principle was

extended to the synthesis of unnatural polysaccharides. In relation to the polyme. mechanism, fundamental mechanistic aspects of in vivo reaction of these enzymes are also discussed. Oligo- and polysaccharide synthesis is achieved by using other classes of enzymes as catalysts, e.g. glycosyltransferases, phosphorylases, and artificial glycosynthases prepd. by ***mutation*** of glycosylases. Conventional chem. syntheses are based on polycondensation, ring-opening polyme., and stepwise elongation. Some characteristics of these syntheses are described.

L12 ANSWER 10 OF 40 CAPLUS COPYRIGHT 2003 ACS

2001:752785 Document No. 135:368464 Improved enantioselectivity of a ***lipase*** by rational protein engineering. Rotticci, Didier; Rotticci-Mulder, Johanna C.; Denman, Stuart; Norin, Torbjorn; Hult, Karl (Department of Chemistry, Organic Chemistry, Royal Inst. of Technology, Stockholm, 10044, Swed.). ChemBioChem, 2(10), 766-770 (English) 2001. CODEN: CBCHX. ISSN: 1439-4227. Publisher: Wiley-VCH Verlag GmbH.

AB A model based on two different binding modes for alc. enantiomers in the active site of a ***lipase*** allowed rational redesign of its enantioselectivity. 1-Halo-2-octanols were poorly resolved by *Candida antarctica* ***lipase*** B. Interactions between the substrates and the ***lipase*** were investigated with mol. modeling. Unfavorable interactions were found between the halogen moiety of the fast-reacting S enantiomers and a region situated at the bottom of the active site (***stereoselectivity*** pocket). The ***lipase*** was virtually mutated in this region and energy contour maps of some variants displayed better interactions for the target substrates. Four selected variants of the ***lipase*** were produced and kinetic resoln. expts. were undertaken with these mutants. Single point ***mutations*** gave rise to one variant with doubled enantioselectivity as well as one variant with annihilated enantioselectivity towards the target halohydrins. An increased vol. of the ***stereoselectivity*** pocket caused a decrease in enantioselectivity, while changes in electrostatic potential increased enantioselectivity. The enantioselectivity of these new ***lipase*** variants towards other types of alc. was also investigated. The changes in enantioselectivity caused by the ***mutations*** were well in agreement with the proposed model concerning the chiral recognition of alc. enantiomers by this ***lipase***.

L12 ANSWER 11 OF 40 CAPLUS COPYRIGHT 2003 ACS

2001:650375 Document No. 135:340802 Rational design of enantioselective enzymes requires considerations of entropy. Ottosson, Jenny; Rotticci-Mulder, Johanna C.; Rotticci, Didier; Hult, Karl (Department of Biotechnology, Royal Institute of Technology, Stockholm, SE-100 44, Swed.). Protein Science, 10(9), 1769-1774 (English) 2001. CODEN: PRCIEI. ISSN: 0961-8368. Publisher: Cold Spring Harbor Laboratory Press.

AB Entropy was shown to play an equally important role as enthalpy for how enantioselectivity changes when redesigning an enzyme. By studying the temp. dependence of the enantiomeric ratio E of an enantioselective enzyme, its differential activation enthalpy (.DELTA.R-S.DELTA.H.dbldag.) and entropy (.DELTA.R-S.DELTA.S.dbldag.) components can be detd. This was done for the resoln. of 3-methyl-2-butanol catalyzed by *Candida antarctica* ***lipase*** B and five variants with one or two point ***mutations***. .DELTA.R-S.DELTA.S.dbldag. was in all cases equally significant as .DELTA.R-S.DELTA.H.dbldag. to E. One variant, T103G, displayed an increase in E, the others a decrease. The altered enantioselectivities of the variants were all related to simultaneous changes in .DELTA.R-S.DELTA.H.dbldag. and .DELTA.R-S.DELTA.S.dbldag.. Although the changes in .DELTA.R-S.DELTA.H.dbldag. and .DELTA.R-S.DELTA.S.dbldag. were of a compensatory nature the compensation was not perfect, thereby allowing modifications of E. Both the W104H and the T103G variants displayed larger .DELTA.R-S.DELTA.H.dbldag. than wild type but exhibited a decrease or increase, resp., in E due to their different relative increase in .DELTA.R-S.DELTA.S.dbldag..

L12 ANSWER 12 OF 40 CAPLUS COPYRIGHT 2003 ACS

2001:622373 Document No. 136:66128 Oligosaccharide binding to family 11 xylanases: both covalent intermediate and mutant product complexes display 2,5B conformations at the active centre. Sabini, Elisabetta; Wilson, Keith S.; Danielsen, Steffen; Schuelein, Martin; Davies, Gideon J. (Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York, YO10 5DD, UK). Acta Crystallographica, Section D:

AB The glycoside ***hydrolase*** sequence-based classification reveals two families of enzymes which hydrolyze the .beta.-1,4-linked backbone of xylan; these xylanase families are termed GH-10 and GH-11. Family GH-11 xylanases are intriguing in that catalysis is performed via a covalent intermediate adopting an unusual 2,5B (boat) conformation, a conformation which also fulfils the ***stereochem*** . constraints of the oxocarbenium ion-like transition state. Here, the 1.9 .ANG. structure of a nucleophile E94A mutant of the Xyn11 from *Bacillus agaradhaerens* in complex with xylotriose is presented. Intriguingly, this complex also adopts the 2,5B conformation in the -1 subsite, with the vacant space provided by the Glu Ala ***mutation*** allowing the sugar to adopt the .alpha.-configuration at C1. The structure of the covalent 2-deoxy-2-fluoroxyllobiosyl-enzyme intermediate has been extended to at. (1.1 .ANG.) resoln.

L12 ANSWER 13 OF 40 CAPLUS COPYRIGHT 2003 ACS

2001:542805 Document No. 135:253567 Enantioselectivity of recombinant *Rhizomucor miehei* ***lipase*** in the ring opening of oxazolin-5(4H)-ones. Turner, Nigel A.; Gaskin, Duncan J. H.; Yagnik, Asutosh T.; Littlechild, Jennifer A.; Vulfson, Evgeny N. (Institute of Food Research, Norwich Research Park, Norwich, NR4 7UA, UK). Protein Engineering, 14(4), 269-278 (English) 2001. CODEN: PRENE9. ISSN: 0269-2139. Publisher: Oxford University Press.

AB Enantioselectivity of enzyme catalysis is often rationalized via active site models. These models are constructed on the basis of comparing the enantiomeric excess of product obsd. in a series of reactions which are conducted with a range of homologous substrates, typically carrying various side chain substitutions. Surprisingly the practical application of these simple but informative pocket size models has been rarely tested in genetic engineering expts. In this paper we report the construction, purifn. and enantioselectivity of two recombinant *Rhizomucor miehei* ***lipases*** which were designed to check the validity of such a model in reactions of ring opening of oxazolin-5(4H)-ones.

L12 ANSWER 14 OF 40 CAPLUS COPYRIGHT 2003 ACS

2001:148501 Document No. 134:291854 Mechanisms of glycosyl transferases and ***hydrolases*** . Withers, S. G. (Protein Engineering Network of Centres of Excellence of Canada and Departments of Chemistry and Biochemistry, The University of British Columbia, Vancouver, BC, V6T 1Z1, Can.). Carbohydrate Polymers, 44(4), 325-337 (English) 2001. CODEN: CAPOD8. ISSN: 0144-8617. Publisher: Elsevier Science Ireland Ltd..

AB A review, with 52 refs. Glycosidases and glycosyl transferases fall into 2 major mechanistic classes; those that hydrolyze the glycosidic bond with retention of anomeric configuration and those that do so with inversion. There are, however, two classes of transferases: those that use nucleotide phosphosugars (NP-sugar-dependent) and those that simply transglycosylate between oligosaccharides or polysaccharides (transglycosylases). The latter are mechanistically similar to retaining glycosidases while the mechanisms of NP-sugar-dependent transferases are far from clear. Retaining glycosidases and the transglycosylases employ a mechanism involving a covalent glycosyl-enzyme intermediate formed and hydrolyzed with acid/base catalytic assistance via oxocarbenium ion-like transition states. This intermediate has been trapped on glycosidases in 2 distinct ways, either by modification of the substrate through fluorination, or of the enzyme through ***mutation*** of key residues. A 3rd method has been developed for trapping the intermediate on transglycosylases involving the use of incompetent substrates that allow formation of the intermediate, but prohibit its transfer as a consequence of their acceptor hydroxyl group being removed. Three-dimensional structures of several of these glycosyl-enzyme complexes, along with those of Michaelis complexes, have been detd. through x-ray crystallog. anal., revealing the identities of important amino acid residues involved in catalysis. In particular they reveal the involvement of the carbonyl oxygen of the catalytic nucleophile in strong hydrogen bonding to the sugar 2-hydroxyl for the .beta.-retainers or in interactions with the ring oxygen for .alpha.-retainers. The glucose ring in the -1 (cleavage) site in the intermediates formed on several cellulases and a .beta.-glucosidase adopts a normal 4C1 chair conformation. By contrast the xylose ring at this site

in a xylanase is substantially distorted into a 2.5B boat conformation, an observation that bears significant ***stereoelectronic*** implications. Substantial distortion is also obsd. in the substrate upon binding to several .beta.-glycosidases, this time to a 1S3 skew boat conformation. Much less distortion is seen in the substrate bound on an .alpha.-transglycosylase. Finally an efficient catalyst for synthesis, but not hydrolysis, of glycosidic bonds has been generated by ***mutation*** of the glutamic acid catalytic nucleophile of a .beta.-glucosidase to an alanine. When used with .alpha.-glucosyl fluoride as a glycosyl donor, along with a suitable acceptor, oligosaccharides up to five sugars in length have been made with yields of up to 90% on individual steps. These new enzymes have been named glycosynthases.

L12 ANSWER 15 OF 40 CAPLUS COPYRIGHT 2003 ACS

2001:93047 Document No. 134:309716 Glycosynthases: new enzymes for oligosaccharide synthesis. Moracci, M.; Trincone, A.; Rossi, M. (Institute of Protein Biochemistry and Enzymology-CNR, Naples, 80125, Italy). Journal of Molecular Catalysis B: Enzymatic, 11(4-6), 155-163 (English) 2001. CODEN: JMCEF8. ISSN: 1381-1177. Publisher: Elsevier Science B.V..

AB The ***mutation*** of putative acid/base and nucleophile of the active sites of retaining glycosyl ***hydrolases***, together with kinetic anal. of the mutants, and ***stereochem***. identification of products lead to useful information for the understanding of the reaction mechanism of these enzymes. This was the preliminary and fundamental step toward the prepn. of new enzymic activities called glycosynthases. A review with 27 refs. Direct exploitation of this information has been possible, leading to the design of four new enzymes for oligosaccharides synthesis. The interest for these biocatalysts rises from the fact that the yield of the reaction can be increased and selectivity can be interpreted as key characteristic of the transfer reaction instead of a balance of hydrolytic and transferring pathways followed either by substrates and products. These new biocatalysts possess different specificities and are promising and useful tools in the construction of oligosaccharide mols. of great biol. interest. This short review focused the attention on different glycosynthases obtained from four glycosyl ***hydrolases*** highlighting on the prepn. and development of these new enzymes.

L12 ANSWER 16 OF 40 CAPLUS COPYRIGHT 2003 ACS

2001:70314 Document No. 134:262797 Substitution of glycine 275 by glutamate (G275E) in ***lipase*** of *Bacillus stearothermophilus* affects its catalytic activity and enantio- and chain length specificity. Kim, Myung Hee; Kim, Hyung-Kwoun; Oh, Byung-Chul; Oh, Tae-Kwang (Environmental Bioresources Lab., Korea Research Institute of Bioscience & Biotechnology, Taejon, 305-600, S. Korea). Journal of Microbiology and Biotechnology, 10(6), 764-769 (English) 2000. CODEN: JOMBES. ISSN: 1017-7825. Publisher: Korean Society for Applied Microbiology.

AB The ***lipase*** gene (lip) from *Bacillus stearothermophilus* was recombined in vitro by utilizing the DNA shuffling technique. After four rounds of shuffling, transformation, and screening based on the initial rate of clear zone formation on a tricaprylin plate, a clone (M10) was isolated, the cell ext. of which showed about 2.8-fold increased ***lipase*** activity. The DNA sequence of the mutant ***lipase*** gene (m10) showed 3 base changes, resulting in two cryptic ***mutations*** and one amino acid substitution; S113 (AGC.fwdarw.AGT), L252 (TTG.fwdarw.TTA), and G275E (GGA.fwdarw.GAA). SDS-PAGE anal. revealed that the increased enzyme activity obsd. in M10 was partly caused by high expression of the m10 ***lipase*** gene. The amt. of the expressed G275E ***lipase*** was estd. to comprise as much as 41% of the total sol. proteins of the cell. The max. velocity (Vmax) of the purified mutant enzyme for the hydrolysis of olive oil was measured to be 3,200 U/mg, which was 10% higher than that of the parental (WT) ***lipase*** (2,900 U/mg). Its optimum temp. for the hydrolysis of olive oil was 68.degree.C and it showed a typical Ca2+-dependent thermostability, properties of which were the same as those of the WT ***lipase***. However, the mutant enzyme exhibited a high enantiospecificity towards (S)-naproxen compared with the WT ***lipase***. In addn., it showed increased hydrolytic activity towards triolein, tricaprin, tricaprylin, and tricaproin.

L12 ANSWER 17 OF 40 CAPLUS COPYRIGHT 2003 ACS
2000:455820 Document No. 133:234317 L-685,458, an Aspartyl ***Protease***
Transition State Mimic, Is a Potent Inhibitor of Amyloid .beta.-Protein
Precursor .gamma.-Secretase Activity. Shearman, Mark S.; Beher, Dirk;
Clarke, Earl E.; Lewis, Huw D.; Harrison, Tim; Hunt, Peter; Nadin, Alan;
Smith, Adrian L.; Stevenson, Graeme; Castro, Jose L. (Departments of
Molecular Biology and Medicinal Chemistry, Merck Sharp & Dohme Research
Laboratories The Neuroscience Research Centre, Harlow, CM20 2QR, UK).
Biochemistry, 39(30), 8698-8704 (English) 2000. CODEN: BICBHW. ISSN:
0006-2960. Publisher: American Chemical Society.

AB Progressive cerebral amyloid .beta.-protein (A.beta.) deposition is
believed to play a central role in the pathogenesis of Alzheimer's disease
(AD). Elevated levels of A.beta.(42) peptide formation have been linked
to early-onset familial AD-causing gene ***mutations*** in the amyloid
.beta.-protein precursor (A.beta.PP) and the presenilins. Sequential
cleavage of A.beta.PP by the .beta.- and .gamma.-secretases generates the
N- and C-termini of the A.beta. peptide, making both the .beta.- and
.gamma.-secretases potential therapeutic targets for AD. The
identity of the A.beta.PP .gamma.-secretase and the mechanism by which the
C-termini of A.beta. are formed remain uncertain, although it has been
suggested that the presenilins themselves are novel intramembrane-cleaving
.gamma.-secretases of the aspartyl ***protease*** class. In this
study we report the identification of L-685,458 as a structurally novel
inhibitor of A.beta.PP .gamma.-secretase activity, with a similar potency
for inhibition of A.beta.(42) and A.beta.(40) peptides. This compd.
contains an hydroxyethylene dipeptide isostere which suggests that it
could function as a transition state analog mimic of an aspartyl
protease. The preferred ***stereochem*** of the
hydroxyethylene dipeptide isostere was found to be the opposite to that
required for inhibition of the HIV-1 aspartyl ***protease***, a factor
which may contribute to the obsd. specificity of this compd. Specific and
potent inhibitors of A.beta.PP .gamma.-secretase activity such as
L-685,458 will enable important advances toward the identification and
elucidation of the mechanism of action of this enigmatic ***protease***
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L12 ANSWER 18 OF 40 CAPLUS COPYRIGHT 2003 ACS
2000:125867 Document No. 132:237244 Synthesis and anti-HIV activity of
cosalane analogues incorporating nitrogen in the linker chain.
Casimiro-Garcia, Agustin; De Clercq, Erik; Pannecouque, Christophe;
Witvrouw, Myriam; Stup, Tracy L.; Turpin, Jim A.; Buckheit, Robert W.,
Jr.; Cushman, Mark (Department of Medicinal Chemistry and Molecular
Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue
University, West Lafayette, IN, 47907, USA). Bioorganic & Medicinal
Chemistry, 8(1), 191-200 (English) 2000. CODEN: BMECEP. ISSN: 0968-0896.
Publisher: Elsevier Science Ltd..

AB Introduction of an amido group or an amino moiety into the alkenyl linker
chain of cosalane provided a new series of analogs. The new compds. were
evaluated as inhibitors of the cytopathic effect of HIV-1 and HIV-2 in
cell culture. The replacement of the 1' and 2' carbons in the linker
chain of cosalane by an amido group was generally tolerated. The length
of the linker chain and the ***stereochem*** of the substituent at
C-3 of the steroid ring had significant effects on the antiviral
activity and potency. Incorporation of an amino moiety into the linker
completely abolished the anti-HIV activity. There are several steps in
the HIV replication cycle that have been proposed as targets for the
development of therapeutic agents. However, currently approved anti-HIV
drugs are only directed against the viral enzymes reverse transcriptase or
protease. Drugs capable of interfering with other steps of the
virus life cycle will be highly valuable in the antiretroviral therapy of
AIDS, as they will have different patterns of resistance ***mutations***
than the drugs currently used clin. In addn., their utilization in
combination with other therapeutic agents could provide more potent drug
"cocktails" capable of completely suppressing virus replication.
Consequently, there is an urgent need for the discovery of clin. useful
anti-HIV agents possessing novel mechanisms of action.

L12 ANSWER 19 OF 40 CAPLUS COPYRIGHT 2003 ACS
2000:34996 Document No. 132:90043 Visualization of enzyme-catalyzed
reactions using pH indicators: rapid screening of ***hydrolase***
libraries for enantioselective enzymes. Moris-Varas, Francisco

(Thermogen, Inc., USA). PCT Int. Appl. WO 2000001842 A2 20000113, 23 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US15400 19990707. PRIORITY: US 1998-91880 19980707; US 1999-125708 19990323.

AB The use of pH indicators to monitor enzyme-catalyzed reactions is described. The formation of acid following an enzyme-mediated reaction, such as hydrolysis, causes a drop in the pH that can be visualized by a change in the color of the indicator-contg. soln. Preferred indicators are those showing a color transition within the operational pH range of the enzyme. Using the present system, the enantioselectivity of enzymes such as ***lipases*** and ***esterases*** can be estd. using single isomers under the same conditions and comparing the color turnover for each one. The method is also useful for application to the hierarchical screening of a library of enzymes.

L12 ANSWER 20 OF 40 CAPLUS COPYRIGHT 2003 ACS

1999:651913 Document No. 132:46673 Directed evolution of an ***esterase*** : screening of enzyme libraries based on pH-Indicators and a growth assay. Bornscheuer, U. T.; Altenbuchner, J.; Meyer, H. H. (Institute for Technical Biochemistry, University of Stuttgart, Stuttgart, 70569, Germany). Bioorganic & Medicinal Chemistry, 7(10), 2169-2173 (English) 1999. CODEN: BMECEP. ISSN: 0968-0896. Publisher: Elsevier Science Ltd..

AB In order to resolve a sterically hindered 3-hydroxy Et ester, which was not accepted as substrate by 20 wild-type ***hydrolases***, a directed evolution of an ***esterase*** from *Pseudomonas fluorescens* (PFE) was performed. ***Mutations*** were introduced using the mutator strain *Epicurian coli* XL1-Red. Enzyme libraries derived from seven ***mutation*** cycles were assayed on minimal media agar plates supplemented with pH indicators (neutral red and crystal violet), thus allowing the identification of active ***esterase*** variants by the formation of a red color caused by a pH decrease due to the released acid. A further selection criteria was introduced by using the corresponding glycerol ester, because release of the carbon source glycerol facilitates growth on minimal media. By this strategy, one double mutant (A209D and L181V) of PFE was identified, which hydrolyzed the 3-hydroxy Et ester in a ***stereoselective*** manner (25% ee for the remaining ester, E.appx.5).

L12 ANSWER 21 OF 40 CAPLUS COPYRIGHT 2003 ACS

1999:503703 Document No. 131:281046 Unique anti-human immunodeficiency virus activities of the nonnucleoside reverse transcriptase inhibitors calanolide A, costatolide, and dihydrocostatolide. Buckheit, Robert W., Jr.; White, E. Lucile; Eliakas-Boltz, Valerie; Russell, Julie; Stup, Tracy L.; Kinjerski, Tracy L.; Osterling, Mark C.; Weigand, Ann; Bader, John P. (Infectious Disease Research Department, Serquest/Southern Research Institute, Frederick, MD, 21701, USA). Antimicrobial Agents and Chemotherapy, 43(8), 1827-1834 (English) 1999. CODEN: AMACCQ. ISSN: 0066-4804. Publisher: American Society for Microbiology.

AB (+)-Canolide A (NSC 650886) has previously been reported to be a unique and specific nonnucleoside inhibitor of the reverse transcriptase (RT) of human immunodeficiency virus (HIV) type 1 (HIV-1). Two isomers of cananolide A, (-)-canolide B (NSC 661122; costatolide) and (-)-dihydrocanolide B (NSC 661123; dihydrocostatolide), possess antiviral properties similar to those of cananolide A. Each of these three compds. possesses the phenotypic properties ascribed to the pharmacol. class of nonnucleoside RT inhibitors (NNRTIs). The cananolide analogs, however, exhibit 10-fold enhanced antiviral activity against drug-resistant viruses that bear the most prevalent NNRTI resistance that is engendered by amino acid change Y181C in the RT. Further enhancement of activity is obsd. with RTs that possess the Y181C change together with ***mutations*** that yield resistance to AZT. In addn., enzymic inhibition assays have demonstrated that the compds. inhibit RT through a mechanism that affects both the Km for dTTP and the Vmax, i.e., mixed-type inhibition. In fresh human cells, costatolide and dihydrocostatolide are highly effective inhibitors of low-passage clin. virus strains, including

those representative of the various HIV-1 clade strains, syncytium-inducing and non-syncytium-inducing isolates, and T-tropic and monocyte-tropic isolates. Similar to calanolide A, decreased activities of the two isomers were obsd. against viruses and RTs with amino acid changes at residues L100, K103, T139, and Y188 in the RT, although costatolide exhibited a smaller loss of activity against many of these NNRTI-resistant isolates. Comparison of cross-resistance data obtained with a panel of NNRTI-resistant virus strains suggests that each of the three ***stereoisomers*** may interact differently with the RT, despite their high degree of structural similarity. Selection of viruses resistant to each of the three compds. in a variety of cell lines yielded viruses with T139I, L100I, Y188H, or L187F amino acid changes in the RT. Similarly, a variety of resistant virus strains with different amino acid changes were selected in cell culture when the calanolide analogs were used in combination with other active anti-HIV agents, including nucleoside and nonnucleoside RT and ***protease*** inhibitors. In assays with combinations of anti-HIV agents, costatolide exhibited synergy with these anti-HIV agents. The calanolide isomers represent a novel and distinct subgroup of the NNRTI family, and these data suggest that a compd. of the calanolide A series, such as costatolide, should be evaluated further for therapeutic use in combination with other anti-HIV agents.

L12 ANSWER 22 OF 40 CAPLUS COPYRIGHT 2003 ACS

1999:238540 Document No. 130:277653 Use of Escherichia coli XL1 Red to change substrate specificity of enzymes. Bornscheuer, Uwe; Meyer, H. H.; Altenbuchner, Johann (BASF A.-G., Germany). Ger. Offen. DE 19743683 A1 19990408, 12 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1997-19743683 19971002.

AB The title method for changing enzyme substrate specificity comprises (1) transforming E. coli XL1 Red with a copy of the enzyme gene, (2) culture of the transformant to mutate the enzyme gene, (3) transfer of the mutated enzyme gene to another microorganism which does not express the enzyme activity, (4) culture of this new transformant in a selection medium contg. a substrate reflective of the desired substrate specificity, and (5) selection of the transformant which has the desired activity. Steps 1-5 may be repeated until the desired substrate specificity is obtained. The procedure was applied to an ***esterase*** gene estF of *Pseudomonas fluorescens*. The mutated enzyme, contg. substitution ***mutations*** A209D and L181V, acquired the ability to ***stereospecifically*** hydrolyze 5-benzyloxy-3-hydroxy-4,4-dimethylpentanoic acid Et ester.

L12 ANSWER 23 OF 40 CAPLUS COPYRIGHT 2003 ACS

1999:34179 Document No. 130:236072 Pharmacogenetics of the 5-lipoxygenase pathway in asthma. Silverman, E.; In, K.-H.; Yandava, C.; Drazen, J. M. (Pulmonary and Critical Care Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA). Clinical and Experimental Allergy, 28(Suppl. 5), 164-170 (English) 1998. CODEN: CLEAEN. ISSN: 0954-7894. Publisher: Blackwell Science Ltd..

AB A review, with 35 refs. It is now well-appreciated that asthma is a chronic inflammatory disease of the airways; among the inflammatory cells that have been implicated in the asthmatic lesion are eosinophils and mast cells. Although these cells have the capacity to produce a no. of distinct chem. mediators, the cysteinyl leukotrienes have recently been identified as important mediators of the asthmatic response. The leukotrienes are derived from arachidonic acid released from membrane phospholipids by the action of phospholipases. The arachidonic acid so released in the presence of the 5-lipoxygenase (5-LO)-activating protein becomes a substrate for the enzyme 5-LO. This enzyme catalyzes the ***stereo*** -specific addn. of mol. oxygen to arachidonic acid to form the product known as leukotriene A4. Leukotriene A4 subsequently becomes a substrate for one of two enzymes, leukotriene A4 epoxide ***hydrolase*** or LTC4 synthase. The former catalyzes the formation of LTB4, whereas the latter catalyzes the formation of the cysteinyl leukotrienes. Thus, the enzyme 5-LO is critically posed to serve as a regulator of leukotriene synthesis. 5-LO action is known to be regulated at a no. of levels; the mechanisms include regulation of action of the mature protein and regulation of 5-LO gene transcription and translation; there is good reason to believe that all forms of 5-LO regulation are highly interdependent. In this regard, the authors describe the presence

and functional consequences of a series of naturally occurring ***mutations*** in 5-LO core promoter. These ***mutations*** modify gene transcription in vitro, and may have functional consequences in vivo.

L12 ANSWER 24 OF 40 CAPLUS COPYRIGHT 2003 ACS
1998:759184 Document No. 129:340876 Biosynthesis of D-amino acid-containing peptides. Exploring the role of peptide isomerases. Volkmann, R. A.; Heck, S. D. (Pfizer Central Research, Groton, CT, 06340, USA). EXS, 85(D-Amino Acids in Sequences of Secreted Peptides of Multicellular Organisms), 87-105 (English) 1998. CODEN: EXSEE7. ISSN: 1023-294X. Publisher: Birkhaeuser Verlag.

AB A review with 65 refs. is given. The discovery of D-amino acid residues in a growing no. of gene-encoded pep-tides suggests that such biochem. modifications are more common than initially thought. In fact, the extent to which o-amino acids are incorporated into peptides by multicellular organisms probably has not been fully realized, since routine Edman sequencing does not provide the abs. ***stereochem*** . of amino acid residues. Unless both the D and L isomers of a particular peptide sequence are isolated, D-amino acid-contg. peptides are often identified only after synthesis of naturally-occurring peptide fails to yield the desired activity. To date, D-amino acid residues (e.g., alanine, methionine, leucine, isoleucine, Ph alanine, asparagine, tryptophan and serine) have been identified in peptides from a variety of species, including frogs, snails, clams, lobsters and spiders. While most have a single D-amino acid residue located near their N-termini, an exception is found with .omega.-Aga IVB. The examples highlighted in this chapter are the result of a unique strategy of multicellular organisms to circumvent ***stereochem*** . limitations imposed by the genetic code in an effort to increase mol. diversity. The presence of D-amino acids permits the generation of novel tertiary structure that could not be accessed from L-amino acids alone. Moreover, advantages of increased potency and ***protease*** stability are often obsd. Our understanding of the biosynthesis of these o-amino acid-contg. peptides is still in its infancy. Nevertheless, the discovery of a novel peptide isomerase from the venom of the *Agelenopsis aperta* spider provides some important clues to explain the incorporation of single D-amino acid residues within a peptide chain. Given its high homol. with other serine ***proteases*** , the isomerase may represent an opportune ***mutation*** in response to evolutionary pressures. Yet, is the isomerase a unique exception or simply the first in a class of enzymes of varying substrate specificity capable of synthesizing n-amino acid-contg. peptides. To be sure, much more remains to be explored about the precise timing and mechanism of the isomerization process, in addn. to obtaining further structural data on the enzyme itself. Therein lies the continuation of this fascinating story in enzyme biochem.

L12 ANSWER 25 OF 40 CAPLUS COPYRIGHT 2003 ACS
1998:601989 Document No. 129:312661 Rational design of *Rhizopus oryzae* ***lipase*** with modified ***stereoselectivity*** toward triradylglycerols. Scheib, H.; Pleiss, J.; Stadler, P.; Kovac, A.; Potthoff, A. P.; Haalck, L.; Spener, F.; Paltauf, F.; Schmid, R. D. (Institute of Technical Biochemistry, University of Stuttgart, Stuttgart, D-70569, Germany). Protein Engineering, 11(8), 675-682 (English) 1998. CODEN: PRENE9. ISSN: 0269-2139. Publisher: Oxford University Press.

AB The binding site of sn-1(3)-regioselective *Rhizopus oryzae* ***lipase*** (ROL) has been engineered to change the ***stereoselectivity*** of hydrolysis of triacylglycerol substrates and analogs. Two types of prochiral triradylglycerols were considered: "flexible" substrates with ether, benzylether or ester groups, and "rigid" substrates with amide or Ph groups, resp., in the sn-2 position. The mol. basis of sn-1(3) ***stereoselectivity*** of ROL was investigated by modeling the interactions between substrates and ROL, and the model was confirmed by exptl. detn. of the ***stereoselectivity*** of wild-type and mutated ROL. For the substrates, the following rules were derived: (i) ***stereopreference*** of ROL toward triradylglycerols depends on the substrate structure. Substrates with "flexible" sn-2 substituents are preferably hydrolyzed at sn-1, "rigid" substrates at sn-3. (ii) ***Stereopreference*** of ROL toward triradylglycerols can be predicted by analyzing the geometry of the substrate docked to ROL: if the torsion angle .PHI.O3-C3 of glycerol is more than 150.degree., the substrate will preferably be hydrolyzed in sn-1, otherwise in sn-3. For ROL, the

following rules were derived: (i) residue 258 affects ***stereoselectivity*** by steric interactions with the sn-2 substituent rather than polar interactions. To a lower extent, ***stereoselectivity*** is influenced by ***mutations*** further apart (L254) from residue 258. (ii) With "rigid" substrates, increasing the size of the binding site (***mutations*** L258A and L258S) shifts ***stereoselectivity*** of hydrolysis toward sn-1, decreasing its size (L258F and L258F/L254F) toward sn-3.

L12 ANSWER 26 OF 40 CAPLUS COPYRIGHT 2003 ACS

1998:561803 Document No. 129:271774 1,3-Dichloropropene Epoxides:

Intermediates in Bioactivation of the Promutagen 1,3-Dichloropropene. Schneider, Manfred; Quistad, Gary B.; Casida, John E. (Environmental Chemistry and Toxicology Laboratory Department of Environmental Science Policy and Management, University of California, Berkeley, CA, 94720-3112, USA). Chemical Research in Toxicology, 11(10), 1137-1144 (English) 1998. CODEN: CRTOEC. ISSN: 0893-228X. Publisher: American Chemical Society.

AB 1,3-Dichloropropene (1,3-D), a major soil fumigant nematicide, is genotoxic in many types of assays, leading to its classification as possibly carcinogenic in humans. This study tests in three steps the hypothesis that 1,3-D is a promutagen activated by epoxidn. and further reaction of the 1,3-D-epoxides. ***Stereospecific*** epoxidn. of 1,3-D (examd. as the cis/trans mixt. and as individual isomers) to the corresponding cis- and trans-1,3-D-epoxides is demonstrated here for the first time, both in vitro in a mouse liver microsome-NADPH system and in vivo in the liver of i.p.-treated mice, using GC/MS for product identification and quantitation. The cis epoxide is obsd. in higher yield than the trans epoxide, both in vitro and in vivo, and the cis isomer also reacts slower than the trans isomer with GSH alone or catalyzed by GSH S-transferase. Cis- and trans-1,3-D-Epoxides are stable in acetone or chloroform but degrade completely in Me2SO exclusively to 2-chloroacrolein (30 min at 40 .degree.C). Epoxide decompn. is slower in pH 7.4 phosphate buffer (t1/2 = 116 and 64 min for cis and trans, resp., at 41 .degree.C) with a >99% yield of 3-chloro-2-hydroxypropanal (and its dimer) and <0.5% formation of 2-chloroacrolein (for which the t1/2 is 248 min at 41 .degree.C). Mutagenicity assays in *Salmonella typhimurium* TA100 (std. plate incorporation) establish high potencies of 37, 17, and 150 revertants/nmol for cis- and trans-1,3-D-epoxides and 2-chloroacrolein, resp. The mutagenicity of the epoxides is due either to their direct action or to a degrdn. product formed at physiol. pH, i.e., 3-chloro-2-hydroxypropanal or its dehydrochlorination products. The candidate mutagens methylglyoxal and glycidaldehyde are not detected as breakdown products of 3-chloro-2-hydroxypropanal at pH 7.4 and also have low mutagenic activity in TA100. It is therefore proposed that the penultimate and ultimate mutagens of 1,3-D metab. are the corresponding epoxides and their direct hydrolysis product 3-chloro-2-hydroxypropanal, resp.

L12 ANSWER 27 OF 40 CAPLUS COPYRIGHT 2003 ACS

1998:466888 Document No. 129:213436 Effect of ***mutations*** in *Candida antarctica* B ***lipase***. Patkar, S.; Vind, J.; Kelstrup, E.; Christensen, M. W.; Svendsen, A.; Borch, K.; Kirk, O. (Enzyme Research, Novo Nordisk A/S, Bagsvaerd, 2880, Den.). Chemistry and Physics of Lipids, 93(1-2), 95-101 (English) 1998. CODEN: CPLIA4. ISSN: 0009-3084. Publisher: Elsevier Science Ireland Ltd..

AB Three variants of the *Candida antarctica* B ***lipase*** have been constructed and characterized. The variant contg. the T103G ***mutation***, which introduces the consensus sequence G-X-S-X-G found in most other known ***lipases***, shows an increased thermostability but retains only half the specific activity of the native enzyme. Also in ester synthesis the activity is lowered but the specificity and enantioselectivity remains unchanged. The W104H mutant, in which more space is introduced into the active site, has more dramatically changed properties. Both the thermostability and the specific activity are slightly reduced but the activity and specificity in ester synthesis is highly different from the native enzyme. In general, the activity is very low and the enantioselectivity is, furthermore, highly reduced. Finally, the ***mutation*** M72L was introduced to increase the oxidn. stability of the enzyme. This variant did exhibit an increased resistance toward oxidn. but the thermostability was, unfortunately, also reduced.

1998:243909 Document No. 129:37944 Directed evolution of an ***esterase*** for the ***stereoselective*** resolution of a key intermediate in the synthesis of epothilones. Bornscheuer, Uwe T.; Altenbuchner, Josef; Meyer, Hartmut H. (Institute for Technical Biochemistry, University of Stuttgart, Stuttgart, 70569, Germany). Biotechnology and Bioengineering, 58(5), 554-559 (English) 1998. CODEN: BIBIAU. ISSN: 0006-3592. Publisher: John Wiley & Sons, Inc..

AB The directed evolution of an ***esterase*** from *Pseudomonas fluorescens* using the mutator strain *Epicurian coil XL1-Red* was investigated. Mutants were assayed for their ability to hydrolyze a sterically hindered 3-hydroxy ester, which can serve as a building block in the synthesis of epothilones. Screening was performed by plating ***esterase*** producing colonies derived from ***mutation*** cycles onto minimal media agar plates contg. indicator substances (neutral red and crystal violet). ***Esterase*** -catalyzed hydrolysis of the 3-hydroxy ester (Et or glycerol ester) was detected by the formation of a red color due to a pH decrease caused by the released acid. ***Esterases*** isolated from pos. clones were used in preparative biotransformations of the Et ester. One variant contg. two ***mutations*** (A209D and L181V) ***stereoselectively*** hydrolyzed the Et ester resulting in 25% ee for the remaining ester.

1998:70202 Document No. 128:227653 Creation of enantioselective biocatalysts for organic chemistry by in vitro evolution. Reetz, Manfred T.; Zonta, Albin; Schimossek, Klaus; Liebeton, Klaus; Jaeger, Karl-Erich (Max-Planck-Inst. Kohlenforschung, Mulheim an der Ruhr, D-45470, Germany). Angewandte Chemie, International Edition in English, Volume Date 1997, 36(24), 2830-2832 (English) 1998. CODEN: ACIEAY. ISSN: 0570-0833. Publisher: Wiley-VCH Verlag GmbH.

AB The authors describe a new approach to developing enantioselective catalysts, namely in vitro evolution. The enantioselective hydrolysis of racemic p-nitrophenyl 2-methydecanoate was chosen as the test reaction. ***Lipase*** from the bacterium *Pseudomonas aeruginosa* was used as the biocatalyst. The ***lipase*** gene was subjected to random mutagenesis using the "error-prone polymerase chain reaction" and the mutants screened for their enantioselectivity in the test reaction.

1997:621074 Document No. 127:304721 ***Mutation*** of tyrosine 383 in leukotriene A4 ***hydrolase*** allows conversion of leukotriene A4 into 5S,6S-dihydroxy-7,9-trans-11,14-cis-eicosatetraenoic acid. Implications for the epoxide ***hydrolase*** mechanism. Andberg, Martina Blomster; Hamberg, Mats; Haeggstrom, Jesper Z. (Department of Medical Biochemistry and Biophysics, Division of Chemistry II, Karolinska Institutet, Stockholm, S-171 77, Swed.). Journal of Biological Chemistry, 272(37), 23057-23063 (English) 1997. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Leukotriene A4 (LTA4) ***hydrolase*** (I) is a bifunctional Zn-contg. metalloenzyme that catalyzes the final step in the biosynthesis of the proinflammatory mediator, leukotriene B4 (LTB4). In previous studies with site-directed mutagenesis on mouse I, the authors identified Tyr-383 as a catalytic amino acid involved in the peptidase reaction. Further characterization of the mutants in position 383 revealed that [Y383H]-I, [Y383F]-I, and [Y383Q]-I catalyzed hydrolysis of LTA4 into a novel enzymic metabolite. From anal. by HPLC and gas chromatog./mass spectrometry of material generated in the presence of H216O or H218O, steric anal. of the OH groups, treatment with soybean lipoxygenase, and comparison with a synthetic std., the novel metabolite was assigned the structure 5S,6S-dihydroxy-7,9-trans-11,14-cis-eicosatetraenoic acid (5S,6S-DHETE). The kinetic parameters for the formation of 5S,6S-DHETE and LTB4 were found to be similar. Also, both activities were susceptible to suicide inactivation and were equally sensitive to inhibition by bestatin. Moreover, from the ***stereochem*** . configuration of the vicinal diol, it could be inferred that 5S,6S-DHETE is formed via an SN1 mechanism involving a carbocation intermediate, which in turn indicates that enzymic hydrolysis of LTA4 into LTB4 follows the same mechanism. Inasmuch as sol. epoxide ***hydrolase*** utilizes LTA4 as a substrate to produce 5S,6R-DHETE, the results also suggest a functional relation between I and xenobiotic epoxide ***hydrolases*** .

1997:560718 Document No. 127:247296 Triacylglycerol composition and structure in genetically modified sunflower and soybean oils. Reske, Johanna; Siebrecht, Jodi; Hazebroek, Jan (Pioneer Hi-Bred International, Inc., Johnston, IA, 50131-1004, USA). Journal of the American Oil Chemists' Society, 74(8), 989-998 (English) 1997. CODEN: JAOC7. ISSN: 0003-021X. Publisher: AOCS Press.

AB Changes in compn. were examd. in oils extd. from genetically modified sunflower and soybean seeds. Improvements were made to the anal. methods to accomplish these analyses successfully. Triacylglycerols (TAG) were sep'd. on two 300 mm .times. 3.9 mm 4.mu. Novapak C18 high-performance liq. chromatog. (HPLC) columns and detected with a Varex MKIII evaporative light-scattering detector. Peaks were identified by coelution with known stds. or by detg. fatty acid compn. of eluted TAG by capillary gas chromatog. (GC). ***Stereospecific*** anal. (fatty acid position) was accomplished by partially hydrolyzing TAG with Et magnesium bromide and immediately derivatizing the resulting diacylglycerols (DAG) with (S)-(+)-1-(1-naphthyl)ethyl isocyanate. The derivatized sn-1,2-DAG were completely resolved from the sn-2,3-DAG on two 25 mm .times. 4.6 mm 3 .mu. silica HPLC columns. The columns were chilled to -20.degree.C to obtain baseline resoln. of collected peaks. The distribution of fatty acids on each position of the glycerol backbone was derived from the fatty acid compns. of the two DAG groups and the unhydrolyzed oil. Results for the sn-2 position were verified by hydrolyzing oils with porcine pancreatic ***lipase***, isolating the resulting sn-2 monoacylglycerols by TLC, and detg. the fatty acid compns. by GC. Results demonstrated that alterations in the total fatty acid compn. of these seed oils are detd. by the concn. of TAG species that contain at least one of the modified acyl groups. As expected, no differences were found in TAG with fatty acid quantities unaffected by the specific ***mutation***. In lieu of direct metabolic or enzymic assay evidence, the authors' positional data are nevertheless consistent with TAG biosynthesis in these lines being driven by the mass action of available acyl groups and not by altered specificity of the acyltransferases, the compds. responsible for incorporating fatty acids into TAG.

1996:751801 Document No. 126:28557 Recombinant preparation of ***lipase*** substitution mutants with altered optical activity. Nakanishi, Juji; Karya, Kinya; Hirose, Yoshihiko; Sasaki, Seiji (Amano Pharma Co Ltd, Japan). Jpn. Kokai Tokkyo Koho JP 08256767 A2 19961008 Heisei, 10 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1996-28640 19960122.

PRIORITY: JP 1995-30093 19950125.

AB A substitution mutant of ***lipase*** of *Pseudomonas* or *Chromobacterium* is prep'd. by site-specific ***mutation***. The mutant with substitutions at 221.fwdarw.Leu, 266.fwdarw.Leu, and 287.fwdarw.Ile exhibits a reversed ***stereo*** specificity as compared with its wild type. The substitutions may also occur at 221.fwdarw.Leu, 265.fwdarw.Leu, and 286.fwdarw.Ile. The mutant is useful in manufg. optically active 1,4-dihydropyridine monocarboxylates. Prepn. of the mutant of ***lipase*** PS of *Pseudomonas cepacia* strain M-12-33 and use of the mutant for changing the optically activity of bis(propionyl oxymethyl)1,4-dihydro-2,6-dimethyl-4-(m-nitrophenyl)-3,5-pyridine dicarboxylate were shown.

1996:708121 Document No. 125:321573 Mannanase A from *Pseudomonas fluorescens* ssp. *cellulosa* is a retaining glycosyl ***hydrolase*** in which E212 and E320 are the putative catalytic residues. Bolam, David N.; Hughes, Neil; Virden, Richard; Lakey, Jeremy H.; Hazlewood, Geoffrey P.; Henrissat, Bernard; Braithwaite, Kerynne L.; Gilbert, Harry J. (Departments of Biological and Nutritional Sciences and Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, UK). Biochemistry, 35(50), 16195-16204 (English) 1996. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB Mannanase A (MANA) from *Pseudomonas fluorescens*, a member of glycosyl ***hydrolase*** family 26, was hyperexpressed in *Escherichia coli* and purified to homogeneity. Anal. of the ***stereochem*** course of mannotetraose hydrolysis by purified MANA showed that the configuration of the anomeric carbon was retained on cleavage of the middle glycosidic

bond. These data suggest that the mannanase hydrolyzes mannooligosaccharides by a double-displacement general acid-base mechanism. By hydrophobic cluster anal. (HCA), two glutamate and two aspartate residues were shown to be conserved in all of the glycosyl ***hydrolase*** family 26 enzymes analyzed. In addn., HCA suggested that family 26 was related to the GH-A clan (families 1, 2, 5, 10, 30, 35, 39, and 42) of (.alpha./.beta.)8-barrel glycosyl ***hydrolases***, which led to the prediction that E320 and E212 constitute the catalytic nucleophile and acid-base residues, resp. To investigate the role of these amino acids, site-directed mutagenesis was used to replace the two aspartates with alanine and glutamate, while the two conserved glutamates were changed to alanine and aspartate. The mutant enzymes were purified and their biochem. properties were analyzed. The data showed that neither the D .fwdarw. A nor the D .fwdarw. E ***mutation*** resulted in a dramatic decrease in enzyme activity, suggesting that the two aspartate residues did not play a pivotal role in catalysis. In contrast, modification of either of the glutamate residues to alanine caused a dramatic decrease in kcat against carob galactomannan, azo-carob galactomannan, mannotetraose and 2,4-dinitrophenyl .beta.-mannobioside (2,4-DNPM). The E320A ***mutation*** did not alter the apparent Km (Km') of MANA against these substrates, while E212A resulted in a 27-fold decrease in Km' against 2,4-DNPM. Pre-steady-state kinetics of 2,4-DNPM hydrolysis by E212A showed that there was a rapid burst of 2,4-dinitrophenol release. CD and fluorescence spectroscopy indicated that there were no significant differences between the structures of the mutant and wild-type forms of MANA. These data are consistent with E212 and E320 constituting the catalytic acid-base and nucleophile residues of MANA, resp.

L12 ANSWER 34 OF 40 CAPLUS COPYRIGHT 2003 ACS

1996:428540 Document No. 125:80337 Conversion of Serine-114 to Cysteine-114 and the Role of the Active Site Nucleophile in Acyl Transfer by Myristoyl-ACP Thioesterase from Vibrio harveyi. Li, Jun; Szittner, Rose; Derewenda, Zygmunt S.; Meighen, Edward A. (Department of Biochemistry, McGill University, Montreal, QC, H3G 1Y6, Can.). Biochemistry, 35(31), 9967-9973 (English) 1996. CODEN: BICAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB The lux-specific myristoyl-ACP thioesterase (LuxD) is responsible for diverting myristic acid into the luminescent system and can function as an ***esterase*** and transferase as well as cleave myristoyl-CoA and other thioesters. The recently elucidated crystal structure of the enzyme shows that it belongs to the .alpha./.beta. ***hydrolase*** family and that it contains a typical catalytic triad composed of Asp211, His241, and Ser114. What is unusual is that the nucleophilic S114 is not contained within the ***esterase*** consensus motif GXSXG although the ***stereochem*** . of the turn involving S114 is almost identical to the nucleophilic elbow found in .alpha./.beta. ***hydrolases*** . In contrast to mammalian thioesterases, deacylation of LuxD was the rate-limiting step, with the level of acylated enzyme formed on reaction with myristoyl-CoA and the pre-steady-state burst of p-nitrophenol on cleavage of p-nitrophenyl myristate both being 0.7 mol/mol. Cold chase expts. showed that the deacylation rate of LuxD corresponded closely to the turnover rate of the enzyme with ester or thioester substrates. Replacement of S114 by a cysteine residue generated a mutant (S114C) that was acylated with the same pH dependence as LuxD but had greatly diminished capacity to transfer acyl groups to water or glycerol. The acyl group could be removed from the S114C mutant by neutral hydroxylamine, showing that a cysteine residue had been acylated. ***Mutation*** of H241 creating the double mutant, S114C.cndot.H241N, decreased acylation of the cysteine residue. These results provide direct kinetic and chem. evidence that S114 is the site of acylation linked to H241 in the charge relay system and have led to the recognition of a more general consensus motif flanking the nucleophilic serine in thioesterases.

L12 ANSWER 35 OF 40 CAPLUS COPYRIGHT 2003 ACS

1995:688810 Document No. 123:163981 Structure of HIV-1 ***protease*** with KNI-272, a tight-binding transition-state analog containing allophenylnorstatine. Baldwin, Eric T.; Bhat, T. Narayana; Gulnik, Sergel; Liu, Beishan; Topol, Igor A.; Kiso, Yoshiaki; Mimoto, Tsutomu; Mitsuya, Hiroaki; Erickson, John W. (Frederick Biomedical Supercomputing Center, SAIC-Frederick, NCI-Frederick Cancer Research and Development

Center, Frederick, MD, 21702, USA). Structure (London), 3(6), 581-90 (English) 1995. CODEN: STRUE6. ISSN: 0969-2126. Publisher: Current Biology.

AB HIV-1 ***protease*** (HIV PR), an aspartic ***protease***, cleaves Phe-Pro bonds in the Gag and Gag-Pol viral polyproteins. Substrate-based peptide mimics constitute a major class of inhibitors of HIV PR presently being developed for AIDS treatment. One such compd., KNI-272, which incorporates allophenylnorstatine (Apns)-thioproline (Thp) in place of Phe-Pro, has potent antiviral activity and is undergoing clin. trials. The structure of the enzyme-inhibitor complex should lead to an understanding of the structural basis for its tight binding properties and provide a framework for interpreting the emerging resistance to this drug. The three-dimensional crystal structure of KNI-272 bound to HIV PR has been detd. to 2.0 .ANG. resoln. and used to analyze structure-activity data and drug resistance for the Arg8.fwdarw.Gln and Ile84.fwdarw.Val ***mutations*** in HIV PR. The conformationally constrained Apns-Thp linkage is favorably recognized in its low energy trans conformation, which results in a sym. mode of binding to the active-site aspartic acids and also explains the unusual preference of HIV PR for the S, or syn, hydroxyl group of the Apns residue. The inhibitor recognizes the enzyme via hydrogen bonds to three bridging water mols., including one that is coordinated directly to the catalytic Asp125 residue. The structure of the HIV PR/KNI-272 complex illustrates the importance of limiting the conformational degrees of freedom and of using protein-bound water mols. for binding potent inhibitors. The binding mode of HIV PR inhibitors can be predicted from the ***stereochem*** . relation between adjacent hydroxyl-bearing and side chain bearing carbon atoms of the P1 substituent. The structure also provides a framework for designing analogs targeted to drug-resistant mutant enzymes.

L12 ANSWER 36 OF 40 CAPLUS COPYRIGHT 2003 ACS

1995:538916 Document No. 123:106071 Cutinase from Fusarium solani pisi Hydrolyzing Triglyceride Analogs. Effect of Acyl Chain Length and Position in the Substrate Molecule on Activity and Enantioselectivity. Mannesse, Maurice L. M.; Cox, Ruud C.; Koops, Bart C.; Verheij, Hubertus M.; de Haas, Gerard H.; Egmond, Maarten R.; van der Hijden, Harry T. W. M.; de Vlieg, Jacob (Department of Enzymology and Protein Engineering, Utrecht University, Neth.). Biochemistry, 34(19), 6400-7 (English) 1995. CODEN: BICBWA. ISSN: 0006-2960. Publisher: American Chemical Society.

AB Triglyceride analogs were synthesized in which one of the primary acyl ester functions has been replaced by an alkyl group and the secondary acyl ester bond has been replaced by an acyl amino bond. The chain length at either position was varied, and both (R)- and (S)-enantiomers of each compd. were synthesized. These pseudo triglycerides contain only one hydrolyzable ester bond, and they are ideally suited to studying the influence of the chain length at the 1-, 2-, and 3-position on ***lipase*** activity and on ***stereopreference***. These substrates were used to characterize cutinase from Fusarium solani pisi. Our results show that the activity of cutinase is very sensitive to the length and distribution of the acyl chains and that the highest activities are found when the chains at positions 1 and 3 contain three or four carbon atoms. The enzyme preferentially hydrolyzes the (R)-enantiomers, but this preference is strongly dependent on the acyl chain length distribution, with (R) over (S) activity ratios varying from about 30 to 1. This enantioselectivity was found in three different assay systems: a mixed micellar, a reverse micellar, and a monolayer study. Our data suggest that at least two alkyl chains of the pseudo triglycerides must be fixed during hydrolysis. Therefore, these substrates were used to characterize mutants of cutinase with ***mutations*** in putative lipid binding domains. Two mutants (A85F and A85W) have increased activities. The results obtained with these mutants suggest an interaction of the acyl chain of the scissile ester bond with a surface loop, comprising residues 80-90, in the enzyme-substrate complex.

L12 ANSWER 37 OF 40 CAPLUS COPYRIGHT 2003 ACS

1995:382025 Document No. 122:239505 Inversion of enantioselectivity in hydrolysis of 1,4-dihydropyridines by point ***mutation*** of ***lipase*** PS. Hirose, Yoshihiko; Kariya, Kinya; Nakanishi, Yuji; Kurono, Yoshiaki; Achiwa, Kazuo (Cent. Res. Lab., Amano Pharmaceutical Co., Ltd., Nishikasugai, 481, Japan). Tetrahedron Letters, 36(7), 1063-6 (English) 1995. CODEN: TELEAY. ISSN: 0040-4039. Publisher: Elsevier.

/ Structure 1 in file .gra /

AB A mutant ***lipase*** differing in three amino acids produced alternate enantiomers when reacted with 1,4-dihydropyridinedicarboxylates. Thus, ***lipase*** PS reacted with dihydropyridinecarboxylates I (R = CH₂OCOEt, CH₂OCOCMe₃) to give the (R) monocarboxylic acids II, whereas a mutant ***lipase*** produced the (S) enantiomer.

L12 ANSWER 38 OF 40 CAPLUS COPYRIGHT 2003 ACS

1989:228116 Document No. 110:228116 Modification of protein stability by introduction of disulfide bridges and prolines: geometric criteria for ***mutation*** sites. Balaji, V. N.; Mobasser, Azita; Rao, Shashidhar N. (Allergan Pharm. Inc., Irvine, CA, 92715, USA). Biochemical and Biophysical Research Communications, 160(1), 109-14 (English) 1989. CODEN: BBRCA9. ISSN: 0006-291X.

AB Geometric parameters are defined to characterize disulfide bridges using x-ray crystal structure data on small mols. and used to suggest replacements of amino acids by cysteines to introduce disulfide bridges to increase thermal stability in proteins. Geometric parameters are also defined to identify target amino acids for replacements by proteins to conserve desired structural attributes in the vicinity of disulfide ***mutations*** leading to further structural and thermal stability of proteins. The geometric criteria are applied to the serine ***protease***, subtilisin, to model ***stereochem***. favorable disulfide mutants without altering the active site geometry, implying conservation of native biol. activity.

L12 ANSWER 39 OF 40 CAPLUS COPYRIGHT 2003 ACS

1984:204772 Document No. 100:204772 Mutagenicity of the enantiomers of the diastereomeric bay-region benz[a]anthracene-3,4-diol 1,2-epoxides in bacterial and mammalian cells. Wood, Alexander W.; Chang, Richard L.; Levin, Wayne; Yagi, Haruhiko; Thakker, Dhiren R.; VanBladeren, Peter J.; Jerina, Donald M.; Conney, Allan H. (Dep. Biochem. Drug Metab., Hoffmann-La Roche Inc., Nutley, NJ, 07110, USA). Cancer Research, 43(12, Pt. 1), 5821-5 (English) 1983. CODEN: CNREA8. ISSN: 0008-5472.

GI

/ Structure 2 in file .gra /

AB Enantiomers of the diastereomeric pair of bay-region benz[a]anthracene-3,4-diol 1,2-epoxides in which the benzylic 4-hydroxyl group and epoxide O are cis (isomer 1) or trans (isomer 2) were evaluated for mutagenic activity in 2 histidine-dependent strains of *Salmonella typhimurium*, as well as in an 8-azaguanine-sensitive Chinese hamster cell line. In strain TA 98 of *S. typhimurium*, the diol epoxide with (1S,2R,3R,4S) abs. configuration [(-)-diol epoxide 2] (I) [80433-81-4] was the most active isomer, although there was <3-fold difference in the mutagenicity of the 4 diol epoxides. However, in strain TA 100 of *S. typhimurium*, the enantiomeric diol-epoxide with (1R,2S,3S,4R) abs. configuration [(+)-diol epoxide 2] [80446-23-7] was the most active diol epoxide and the 2 isomers with (3S,4R) abs. configuration [(-)-diol epoxide 1 [80433-78-9] and (+)-diol epoxide 2 [80433-79-0]] were 3-8-fold more active than were the 2 isomers with (3R,4S) configuration. The highest degree of sensitivity to abs. configuration was obsd. in Chinese hamster V-79 cells, in which the (1R,2S,3S,4R) isomer [(+)-diol-epoxide 2] was 3-20-fold more mutagenic than were the other 3 isomers. This metabolically predominant (+)-diol epoxide 2 isomer, which has high activity in strain TA 100 of *S. typhimurium* and the Chinese hamster V-79 cells, has the same abs. configuration as do the bay region diol epoxide isomers of benzo[a]pyrene and chrysene that were shown previously to be exceptionally mutagenic to mammalian cells and highly tumorigenic in mice. Anal. of the mutagenic activity of the (+)- and (-)-isomers of the 1,2- and 3,4-tetrahydroepoxides of benz[a]anthracene revealed only small enantiomeric

differences in strain TA 98 of *S. typhimurium* (2.5-fold) and little, if any, differences (<1.5-fold) in the other 2 mutagenicity systems. However, the extent to which the 4 tetrahydroepoxides were converted to nonmutagenic products by homogeneous microsomal epoxide ***hydrolase*** (EC 3.3.2.3) [9048-63-9] indicated marked differences in the ***stereoselectivity*** of the enzyme. (--)-(3R,4S)-Epoxy-1,2,3,4-tetrahydrobenz[a]anthracene [89618-18-8] appears to be an exceptionally good substrate for epoxide ***hydrolase***.

L12 ANSWER 40 OF 40 CAPLUS COPYRIGHT 2003 ACS

1977:565989 Document No. 87:165989 Optical resolution of menthols and related compounds. Part IV. Asymmetric hydrolysis of dl-menthyl acetate by *Rhodotorula mucilaginosa*. Yamaguchi, Yuzo; Komatsu, Akira; Moroe, Tatsuo (Cent. Res. Lab., Takasago Perfum. Co., Ltd., Tokyo, Japan). Nippon Nogei Kagaku Kaishi, 51(7), 411-16 (Japanese) 1977. CODEN: NNKAA. ISSN: 0002-1407.

AB Culture media contg. roasted soybean flour, yeast ext., or corn steep liquor were suitable for the ***esterase*** formation. Optimum temp. for growth and the asymmetric hydrolysis were 27-29.degree. and 32-33.degree., resp. Optimum pH for growth and for the reaction were 7 and 7.5, resp. Strain improvement was conducted with single cell isolation and UV ***mutation***, by which the activity was increased about 70%. A total of 44.4 g of L-menthol [2216-51-5] was produced from DL-menthyl acetate [29066-34-0] mixt. in 24 h in 1 L culture medium.

=> S REGIO?

L13 1126782 REGIO?

=> S L13 AND L6

L14 52433 L13 AND L6

=> S L14 AND (L1,L2,L3,L4)

L15 1328 L14 AND ((L1 OR L2 OR L3 OR L4))

=> S PCR;S L16 AND L7

117553 PCR

650 PCRS

L16 117659 PCR

(PCR OR PCRS)

L17 15 L16 AND L7

=> S L17 NOT L12

L18 11 L17 NOT L12

=> D 1-11 CBIB ABS

L18 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2003 ACS

2003:251379 Directed evolution of N-acetylneurameric acid aldolase to catalyze enantiomeric aldol reactions. Wada, Masaru; Hsu, Che-Chang; Franke, Dirk; Mitchell, Michael; Heine, Andreas; Wilson, Ian; Wong, Chi-Huey (Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037, USA). Bioorganic & Medicinal Chemistry, 11(9), 2091-2098 (English) 2003. CODEN: BMECEP. ISSN: 0968-0896. Publisher: Elsevier Science Ltd..

AB Expanding the scope of substrate specificity and ***stereoselectivity*** is of current interest in enzyme catalysis. Using error-prone ***PCR*** for in vitro directed evolution, the Neu5Ac aldolase from *Escherichia coli* has been altered to improve its catalytic activity toward enantiomeric substrates including N-acetyl-l-mannosamine and l-arabinose to produce l-sialic acid and l-KDO, the mirror-image sugars of the corresponding naturally occurring d-sugars. The first generation variant contg. two ***mutations*** (Tyr98His and Phe115Leu) outside the (.alpha.,.beta.)8-barrel active site exhibits an inversion of enantioselectivity toward KDO and the second generation variant contains an addnl. amino acid change Val251Ile outside the .alpha.,.beta.-barrel active site that improves the enantiomeric formation of l-sialic acid and l-KDO. The X-ray structure of the triple mutant epNanA.2.5 at 2.3 Å resoln. showed no significant difference between the wild-type and the

mutant enzymes. We probed the potential structural 'hot spot' of enantioselectivity with satn. mutagenesis at Val251, the mutated residue most proximal to the Schiff base forming Lys165. The selected variant had an increase in *k_{cat}* via replacement with another hydrophobic residue, leucine. Further sampling of a larger sequence space with error-prone ***PCR*** selected a third generation variant with significant improvement in L-KDO catalysis and a complete reversal of enantioselectivity.

L18 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2003 ACS

2003:28590 Enhancement of the thermostability and catalytic activity of D-***stereospecific*** amino-acid amidase from *Ochrobactrum anthropi* SV3 by directed evolution. Komeda, Hidenobu; Ishikawa, Naoyoshi; Asano, Yasuhisa (Biotechnology Research Center, Toyama Prefectural University, Kosugi, Toyama, 939-0398, Japan). *Journal of Molecular Catalysis B: Enzymatic*, 21(4-6), 283-290 (English) 2003. CODEN: JMCEF8. ISSN: 1381-1177. Publisher: Elsevier Science B.V..

AB D-Amino-acid amidases, which catalyze the ***stereospecific*** hydrolysis of D-amino-acid amide to yield D-amino acid and ammonia, have attracted increasing attention as catalysts for ***stereospecific*** prodn. of D-amino acids. We screened for the enzyme variants with improved thermostability generated by a directed evolution method with the goal of the application of evolved enzyme to the prodn. of D-amino acids. Random mutagenesis by error-prone ***PCR*** and a filter-based screening was repeated twice, and as a result the most thermostable mutant BFB40 was obtained. Gene anal. of the BFB40 mutant indicated that the mutant enzyme had K278 M and E303 V ***mutations***. To compare the enzyme characteristics with the wild-type enzyme, the mutant enzyme, BFB40, was purified from the *Escherichia coli* (E. coli) transformant. Both the thermostability and apparent optimum temp. of the BFB40 were shifted upward by 5.degree.C compared with those of the wild-type enzyme. The apparent *K_m* value for D-phenylalaninamide of BFB40 enzyme was almost the same with that of the wild-type enzyme, whereas *V_{max}* value was enhanced about three-fold. Almost complete hydrolysis of D-phenylalaninamide was achieved in 2 h from 1.0 M of racemic phenylalaninamide-HCl using the cells of E. coli transformant expressing BFB40 enzyme, the conversion of which was 1.7-fold higher than the case using cells expressing wild-type enzyme after the same reaction time.

L18 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2003 ACS

2002:675778 Document No. 137:213253 Selection by mirror image display. Wong, Chi-Huey (The Scripps Research Institute, USA). PCT Int. Appl. WO 2002067860 A2 20020906, 46 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US5193 20020222.

PRIORITY: US 2001-PV271377 20010222.

AB Non-naturally occurring binders to cell surface carbohydrates and sugars are identified by a screening process that employs immobilized enantiomers of such cell surface carbohydrates and sugars. Preferred non-naturally occurring binders include D-peptides and L-nucleic acids and are resistant to enzymic degrdn. and clearance. Single-chain Fab sequences that bind to sialic acid and KDO in nano-molar affinity were identified by this process. Exemplary screening procedures employed D-KDO, L-sialic acid and an L-sialo-disaccharide have been attached to a solid support for selection of high-affinity binders.

L18 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2003 ACS

2002:481289 Document No. 137:227963 Formation of Benzo[a]pyrene Diol Epoxide-DNA Adducts at Specific Guanines within K-ras and p53 Gene Sequences: Stable Isotope-Labeling Mass Spectrometry Approach. Tretyakova, Natalia; Matter, Brock; Jones, Roger; Shallop, Anthony (University of Minnesota Cancer Center, Minneapolis, MN, 55455, USA). *Biochemistry*, 41(30), 9535-9544 (English) 2002. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB The mutagenicity of a prominent tobacco carcinogen, benzo[a]pyrene

(B[a]P), is believed to result from chem. reactions between its diol epoxide metabolite, (+)-anti-7r,8t-dihydroxy-c9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE), and DNA, producing promutagenic lesions, e.g., (+)-trans-anti-7R,8S,9S-trihydroxy-10S-(N2-deoxyguanosyl)-7,8,9,10-tetrahydrobenzo[a]pyrene (N2-BPDE-dG). Previous studies used the DNA repair enzyme UvrABC endonuclease in combination with ligation-mediated ***PCR*** (LMPCR) to demonstrate an increased reactivity of BPDE toward guanine nucleobases within codons 157, 248, and 273 of the p53 tumor suppressor gene (M. F. Denissenko, et al.). These sites are also "hot spots" for ***mutations*** obsd. in lung tumors of smokers, suggesting an involvement of B[a]P in the initiation of lung cancer. However, the LMPCR approach relies on the ability of the repair enzyme to excise BPDE-induced lesions, and thus the slowly repaired lesions may escape detection. Furthermore, BPDE-DNA adduct structure and ***stereochem*** cannot be detd. In the present work, we performed a direct quant. anal. of N2-BPDE-dG originating from specific guanine nucleobases within p53- and K-ras-derived DNA sequences by using a stable isotope labeling-mass spectrometry approach recently developed in our lab. ¹⁵N-labeled dG was placed at defined positions within DNA sequences derived from the K-ras proto-oncogene and p53 tumor suppressor gene, the two genes most frequently mutated in smoking-induced lung cancer. ¹⁵N-labeled DNA was annealed to the complementary strands, followed by BPDE treatment and liq. chromatog.-electrospray ionization tandem mass spectrometry anal. (HPLC-ESI-MS/MS) of N2-BPDE-dG lesions. The extent of adduct formation at ¹⁵N-labeled guanine was detd. directly from the HPLC-ESI-MS/MS peak area ratios of ¹⁵N-N2-BPDE-dG and N2-BPDE-dG. BPDE-induced guanine adducts were produced nonrandomly along K-ras and p53 gene-derived DNA sequences, with over 5-fold differences in adduct formation depending on sequence context. N-BPDE-dG yield was enhanced by the presence of 5-Me substituent at the cytosine base-paired with the target guanine nucleobase, an endogenous DNA modification characteristic for CpG dinucleotides within the p53 gene. In the K-ras-derived DNA sequence, the majority of N2-BPDE-dG adducts originated from the first position of the codon 12 (GGT), consistent with the large no. of G .fwdarw. T transversions obsd. at this nucleotide in smoking-induced lung cancer. On the contrary, the pattern of N2-BPDE-dG formation within the p53 exon 5 sequences did not correlate with the mutational spectrum in lung cancer, suggesting that factors other than N2-BPDE-dG formation are responsible for these ***mutations***. The stable isotope labeling HPLC-ESI-MS/MS approach described in this work is universally applicable to studies of modifications to isolated DNA by other carcinogens and alkylating drugs.

L18 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2003 ACS

2001:767270 Document No. 135:316963 Rett syndrome genetic diagnosis by detecting ***mutations*** in methyl-CpG-binding protein 2 (MECP2) gene. Yamakawa, Kazuhiro (Institute of Physical and Chemical Research, Japan). Jpn. Kokai Tokkyo Koho JP 2001292775 A2 20011023, 14 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 2000-109638 20000411.

AB A method and reagent kit for allele-specific ***PCR*** based genetic diagnosis for Rett syndrome by detection of ***mutations*** in methyl-CpG-binding protein 2 (MECP2) gene, are disclosed. The kit comprises allele-specific primers. A variety of ***mutations*** in MeCP2 gene was obsd. in Rett syndrome patients. Rett syndrome (RTT, MIM 312750) is a progressive neurodevelopmental disorder and one of the most common causes of mental retardation in females, with an incidence of 1 in 10,000-15,000. Patients with classic RTT appear to develop normally until 6-18 mo of age, then gradually lose speech and purposeful hand use, and develop microcephaly, seizures, autism, ataxia, intermittent hyperventilation and ***stereotypic*** hand movements. After initial regression, the condition stabilizes and patients usually survive into adulthood. As RTT occurs almost exclusively in females, it has been proposed that RTT is caused by an X-linked dominant ***mutation*** with lethality in hemizygous males. Previous exclusion mapping studies using RTT families mapped the locus to Xq28. Using a systematic gene screening approach, the authors have identified ***mutations*** in the gene (MECP2) encoding X-linked methyl-CpG-binding protein 2 (MeCP2) as the cause of some cases of RTT. MeCP2 selectively binds CpG dinucleotides in the mammalian genome and mediates transcriptional repression through interaction with histone deacetylase and the corepressor SIN3A.

L18 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2003 ACS

2000:404498 Document No. 133:294792 Splice site ***mutation*** causing a seven amino acid Notch3 in-frame deletion in CADASIL. Joutel, A.; Chabriat, H.; Vahedi, K.; Domenga, V.; Vayssiere, C.; Ruchoux, M. M.; Lucas, C.; Leys, D.; Bousser, M. G.; Tournier-Lasserve, E. (INSERM U25, Faculte de Medecine Necker-Enfants Malades, Paris, Fr.). Neurology, 54(9), 1874-1875 (English) 2000. CODEN: NEURAI. ISSN: 0028-3878. Publisher: Lippincott Williams & Wilkins.

AB Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an increasingly recognized cause of subcortical ischemic stroke, attacks of migraine with aura, and vascular dementia in human adults. CADASIL is an autosomal dominant condition caused by ***mutations*** within the Notch3 gene. Patients carry highly ***stereotyped*** missense ***mutations*** leading to an odd no. of cysteine residues within one of the epidermal growth factor (EGF)-like repeats of Notch3 extracellular domain. We report on the first splice site ***mutation*** occurring in a somewhat peculiar CADASIL family exhibiting prominent migraine with aura features. This ***mutation*** results in an in-frame deletion of seven amino acids, including one cysteine within the second EGF domain. Family and patients. The main features of this family have been previously reported. In summary, nine subjects presented with white matter abnormalities (WMA) on T2-weighted images; among these, two were asymptomatic and seven were symptomatic. Symptoms included migraine with aura in six patients and progressive dementia in one patient. WMA were assocd. with well delineated hyposignals on T1-weighted image, highly suggestive of deep infarcts in the seven symptomatic subjects. Chromosome 19 linkage anal. data strongly suggested linkage to the CADASIL locus. Since this report, an addnl. patient has become progressively demented and had a stroke. Postmortem ultrastructural examn. in one patient disclosed vascular smooth muscle cell changes identical to those described in CADASIL patients. Mol. genetics investigation. Mutational anal. of the Notch3 gene was performed as previously described. Total RNA was prep'd. from the muscle of one patient and an unrelated control individual, and reverse transcribed according to the manufacturer's instructions (Life Technologies, Cergy Pontoise, France). ***PCR*** amplification was performed on complementary DNA (cDNA) with forward primer 5'-TCTGCCAGAGTCAGT-3', located within exon 3, and reverse primer 5'-TTGAGACATCGGTGTC-3', located within exon 5. Amplicons were cloned into a pGEMT Easy Vector (Promega Inc., Madison, WI). For each piece of amplified cDNA, at least 10 randomly selected clones were sequenced on both strands. Sequence variations were considered only if present in more than one clone. Results: Notch3 mutational scanning in this family revealed an A-to-G transition in one allele within the 3' acceptor splice of exon 4 (figure). Because (1) no other ***mutation*** was detected in the remaining exons, (2) this ***mutation*** was not obsd. in more than 400 control chromosomes, and (3) the ***mutation*** segregated with the neuroradiol. phenotype, we concluded that it is the causative ***mutation*** in this family. Interestingly, the three family members presenting with migraine and having normal MRI results did not carry the ***mutation***. To investigate whether this 3' acceptor splice site ***mutation*** would affect splicing of exon 4, we analyzed reverse-transcribed (RT- ***PCR***) mRNA (mRNA) extd. from muscle. Muscle was chosen because the Notch3 transcript is undetectable in peripheral blood leukocytes of both control subjects and patients (A.J., unpublished results). Nucleotide sequence anal. of the cloned RT- ***PCR*** products from the patient revealed an in-frame 21-base pair deletion in exon 4, from nucleotides G419 to G439, in approx. 50% of the clones. This deletion most likely results from the use of a cryptic 3' acceptor splice site at position 438-439 within exon 4. This ***mutation*** is predicted to lead to an in-frame deletion of seven amino acids (glycine, proline, aspartic acid, cysteine, serine, leucine, proline) in the Notch3 protein, including the sixth cysteine residue of the second EGF domain. Discussion. This is the first report of a splice site ***mutation*** within Notch3. This unusual ***mutation*** could account for the peculiar phenotypic spectrum obsd. within this family; namely, the rarity of stroke events, the high prevalence of migraine with aura, and the occurrence in some patients of very unusual confusion or coma episodes, or both. In this family, CADASIL diagnosis was initially suspected chiefly based on neuroimaging data and not on the clin. features; at that time, none of the patients experienced a stroke event. Interestingly, we found that three patients with migraine and

normal MRI results did not carry the ***mutation*** . This finding, which is likely explained by the high prevalence of migraine within the general population, may be of importance for genetic counseling, given the severity of the prognosis of this disorder. All previously reported Notch3 ***mutations*** result in an odd no. of cysteine residues within an EGF repeat. Importantly, this small in-frame deletion includes one cysteine residue. Our finding provides addnl. evidence that the unpaired cysteine residue, which may promote the abnormal oligomerization of the Notch3 receptor, is likely to play a key role in the pathophysiol. of CADASIL.

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2000:95184 Document No. 132:275353 Diallyl sulfide enhances azoxymethane-induced preneoplasia in Fischer 344 rat colon. Delker, D. A.; Papanikolaou, A.; Suhr, Y.-J.; Rosenberg, D. W. (Toxicology Program, University of Connecticut, Storrs, CT, USA). *Chemico-Biological Interactions*, 124(3), 149-160 (English) 2000. CODEN: CBINA8. ISSN: 0009-2797. Publisher: Elsevier Science Ireland Ltd..

AB Azoxymethane (AOM) is an indirect-acting colon carcinogen that produces a high incidence of precancerous lesions, referred to as aberrant crypt foci (ACF), in rats. This study was undertaken to det. whether high-dose gavage administration of the cytochrome P 450 2E1 (CYP2E1) inhibitor and chemopreventive agent, diallyl sulfide, would reduce the incidence and severity of ACF formation in the distal colons of AOM-treated Fischer 344 rats. Seven-week-old male rats received 150 or 50 mg/kg diallyl sulfide by gavage 24 and 2 h prior to 2 weekly i.p. injections of AOM (20 mg/kg). Ten weeks after the last injection of AOM the rats were sacrificed and the colons removed and stained with 0.2% methylene blue. ACF were visualized using ***stereomicroscopy*** . Rats pretreated with diallyl sulfide exhibited a significant increase in the no. of ACF/cm in the distal colon compared with rats receiving AOM alone. This increase in ACF no. was seen in ACF of all sizes. To examine the effects of diallyl sulfide on the initiation stage of AOM-induced carcinogenesis, ***mutations*** in the K-ras proto-oncogene were also investigated. ACF and normal appearing colonic mucosa (0.2-0.5 mm³) were microdissected for subsequent ***PCR*** -RFLP anal. of a codon 12 (GGT-GGA) activating ***mutation*** in the K-ras gene. Greater than 90% of ACF from AOM-treated animals, regardless of diallyl sulfide treatment, exhibited activating K-ras ***mutations*** . K-ras ***mutations*** were also detected in normal appearing mucosa of AOM-treated animals, although at a lesser frequency (15-35%). These studies demonstrate that diallyl sulfide given in large gavage doses enhances AOM-induced preneoplasia in rats and suggests that diallyl sulfide may alter the disposition of AOM intermediates and(or) enhance colonic promotional activity in the rat.

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1997:705709 Document No. 127:341361 Genetic association between sensitivity to warfarin and expression of CYP2C9*3. Steward, Daniel J.; Haining, Robert L.; Henne, Kirk R.; Davis, George; Rushmore, Thomas H.; Trager, William F.; Rettie, Allan E. (Department of Medicinal Chemistry, University of Washington, Seattle, WA, 98195, USA). *Pharmacogenetics*, 7(5), 361-367 (English) 1997. CODEN: PHMCEE. ISSN: 0960-314X. Publisher: Chapman & Hall.

AB Cytochrome P 450 2C9 (CYP2C9) is largely responsible for terminating the anticoagulant effect of racemic warfarin via hydroxylation of the pharmacol. more potent S-enantiomer to inactive metabolites.

Mutations in the CYP2C9 gene result in the expression of three allelic variants, CYP2C9*1, CYP2C9*2 and CYP2C9*3. Both CYP2C9*2 and CYP2C9*3 exhibit altered catalytic properties in vitro relative to the wild-type enzyme. In the present study, a patient was genotyped who had proven unusually sensitive to warfarin therapy and could tolerate no more than 0.5 mg of the racemic drug/day. ***PCR*** -amplification of exons 3 and 7 of the CYP2C9 gene, followed by restriction digest or sequence anal., showed that this individual was homozygous for CYP2C9*3. In addn., patient plasma warfarin enantiomer ratios and urinary 7-hydroxywarfarin enantiomer ratios were detd. by chiral-phase high performance liq. chromatog. to investigate whether either parameter might be of diagnostic value in place of a genotypic test. Control patients receiving 4-8 mg warfarin/day exhibited plasma S:R ratios of 0.50:1, whereas the patient on very low-dose warfarin exhibited an S:R ratio of 3.9:1. In contrast, the urinary 7-hydroxywarfarin S:R ratio of 4:1 showed the same

stereoselectivity as that reported for control patients. Therefore, expression of CYP2C9*3 is assocd. with diminished clearance of S-warfarin and a dangerously exacerbated therapeutic response to normal doses of the racemic drug. Anal. of the plasma S:R warfarin ratio may serve as a useful alternative test to genotyping for this genetic defect.

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1996:118153 Document No. 124:195303 Genetic polymorphism of CYP2C9 and its effect on warfarin maintenance dose requirement in patients undergoing anticoagulation therapy. Furuya, Hirokazu; Fernandez-Salguero, Pedro; Gregory, Wendy; Taber, Heather; Steward, Annette; Gonzalez, Frank J.; Idle, Jeffrey R. (Laboratory of Molecular Carcinogenesis, NCI, Bethesda, MD, 20892, USA). Pharmacogenetics, 5(6), 389-92 (English) 1995. CODEN: PHMCEE. ISSN: 0960-314X. Publisher: Chapman & Hall.

AB A single amino acid substitution of cysteine for arginine at position 144 in the CYP2C9 protein, arising from a base substitution, gives rise to an allelic variant of CYP2C9 termed R144C. It has been found that the ***mutation*** markedly affected neither the regio- nor the ***stereoselectivity*** of warfarin hydroxylation. Expression of the variant cDNA revealed a profound effect of this single amino acid change. It should be expected therefore that the R144C allele would affect the metab. of (S)-warfarin *in vivo*. In order to examine this proposition, the authors have investigated the CYP2C9 genotype by ***PCR*** in a series of patients attending an anticoagulation clinic for routine monitoring and related these findings to their individual warfarin dose requirement for stable anticoagulation.

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1994:2562 Document No. 120:2562 Complementary tumor induction in neural grafts exposed to N-ethyl-N-nitrosourea and an activated myc gene. Bruestle, O.; Petersen, I.; Radner, H.; Hoell, T.; Plate, K. H.; Kleihues, P.; Wiestler, O. D. (Inst. Neuropathol., Univ. Zurich, Zurich, CH-8091, Switz.). Carcinogenesis, 14(8), 1715-18 (English) 1993. CODEN: CRNGDP. ISSN: 0143-3334.

AB Using a combination of transplacental carcinogen exposure and retrovirus-mediated oncogene transfer into fetal brain transplants, the authors have studied complementary transformation by N-ethyl-N-nitrosourea (NEU) and the v-myc oncogene in the nervous system. Previous expts. had demonstrated that both agents will not induce tumors independently whereas simultaneous expression of v-H-ras and v-gag/myc exerted a powerful transforming potential in neural grafts. In order to identify other genetic alterations that co-operate with an activated myc gene, the neurotropic carcinogen NEU was used to generate ***mutations*** of cellular genes. On embryonic day 14 (ED14), pregnant donor animals (F344 rats) received a single i.v. dose of NEU (50 mg/kg). Twenty-four hours later (ED15), the fetal brains were removed, triturated, and incubated with a retroviral vector carrying the v-gag/myc oncogene. Subsequently, these primary cell suspensions were transplanted ***stereotactically*** into the caudate-putamen of syngenic adult recipients. After latency periods of 3-6 mo, 5 of 10 recipients harboring ED15 fetal brain transplants developed malignant, poorly differentiated neuroectodermal tumors in the grafts. No tumor development was obsd. in 7 recipients harboring ED16 neural grafts. Cell lines were established from 3 tumors and the 110-kd gag/myc fusion protein encoded by the retroviral construct was identified in the tumors by Western blotting. Several candidate genes for mutational activation by NEU including the H-ras, K-ras, and neu oncogenes were analyzed for specific point ***mutations*** by polymerase chain reaction (***PCR***) and direct DNA sequencing of the ***PCR*** products. However, no ***mutations*** were found in any of these genes. These findings lend further support to the multistep hypothesis of neoplastic transformation in the brain. The tumors induced in this model provide an interesting tool for the identification of genes that cooperate with an activated myc gene in neurocarcinogenesis.

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1991:625872 Document No. 115:225872 Mutational activation of c-Ha-ras genes in intraductal proliferation induced by N-nitroso-N-methylurea in rat mammary glands. Sakai, Hirotsuka; Ogawa, Katsuhiro (Dep. Pathol., Asahikawa Med. Coll., Asahikawa, 078, Japan). International Journal of Cancer, 49(1), 140-4 (English) 1991. CODEN: IJCNAW. ISSN: 0020-7136.

AB Although administration of a single dose of N-nitroso-N-methylurea (NMU)

to young virgin rats induces a high rate of mammary carcinomas, precise histogenesis of the carcinomas has not been well characterized. In this study, the authors investigated the alterations of H-ras gene in early focal lesions as well as carcinomas in the mammary glands of F344 rats treated with NMU. At 2 wk after treatment, intraductal proliferation (IDP) was occasionally obsd., and mammary carcinomas emerged at 12 and 36 wk. The individual lesions of IDP and carcinomas were scooped out from the tissue sections under a ***stereomicroscope***, and the DNA-sequence-spanning codon 12 of H-ras gene was amplified from the tissue sections by polymerase chain reaction (***PCR***). The anal. of amplified DNA by oligonucleotides hybridization had a point ***mutation*** (G-to-A transition) at the 2nd position of H-ras codon 12. However, the DNA amplified from the areas, which appear histol. normal, never showed such ***mutation***. The results indicate that IDP is a very early change for NMU-induced mammary carcinogenesis.

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